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# VIOLENT SPORE-DISCHARGE IN *TILLETIA TRITICI*

*Tilletia Tritici* and *T. laevis* are fungi which cause the well-known stinking smut disease or bunt of wheat. An affected wheat grain becomes transformed into a sack filled with several million, closely packed, minute, brown chlamydospores which, in the mass, have the odour of decaying fish. These spores become powdery and, under natural conditions, are passively dispersed by wind and rain. In the artificial process of threshing, the smut-balls are broken open and much of the chlamydospore-powder becomes adherent to the outer surface of the sound wheat grains. When smutty grains are planted in the soil, the fungus attacks the wheat seedlings and once more gives rise to the smut disease. Farmers in Canada and other countries treat the seed-wheat with formalin or some other poisonous substance. This kills the chlamydospores and greatly reduces the incidence of the disease.

When a chlamydospore germinates on a wet substratum (Fig. 1, A), it gives rise to a short germ-tube or *basidium* (promycelium) which at its apex produces aerially a crown of from four to sixteen slender rod-shaped structures, called by Brefeld and others *primary conidia*. These conidia have been regarded by botanists in general as morphologically equivalent to the basidiospores of mushrooms and of rust fungi.[1]

The primary conidia, often while still on the basidium as illustrated by Brefeld,[2] conjugate in pairs, each pair forming an H-shaped structure. The work of Rawitscher[3] has shown that, during conjugation, a nucleus present in one of the two conidia passes via the bridging hypha into the other conidium, so that this becomes binucleate. The primary conidia readily become detached from the basidium-body which has borne them, but they are never discharged by that organ in a violent manner.

Each H-shaped pair of primary conidia, while still attached to the basidium-body, or after becoming detached, may put out a short sterigma at the end of which there is developed a sickle-shaped spore, called by Brefeld and others a *secondary conidium* (Fig. 1, A). Or, if malt-agar or other suitable nutrient medium be

[1] Cf. F. L. Stevens, "Plant Disease Fungi," New York, 1925, p. 213.
[2] O. Brefeld, "Untersuchungen über Pilze," Heft 5, 1883, Taf. 12, Figs. 26 and 27.
[3] F. Rawitscher, "Zur Sexualität der Brandpilze *Tilletia Tritici*," *Ber. d. Deutsch. Bot. Ges.*, Bd. 32, 1914, p. 310.

supplied, each H-shaped pair of primary conidia may put out a germ-tube which branches and rebranches for a long time and produces singly, at intervals along its hyphæ, many scores or hundreds of the sickle-shaped secondary conidia (Fig. 1, B). Each sickle-shaped conidium is produced aerially on a short conical

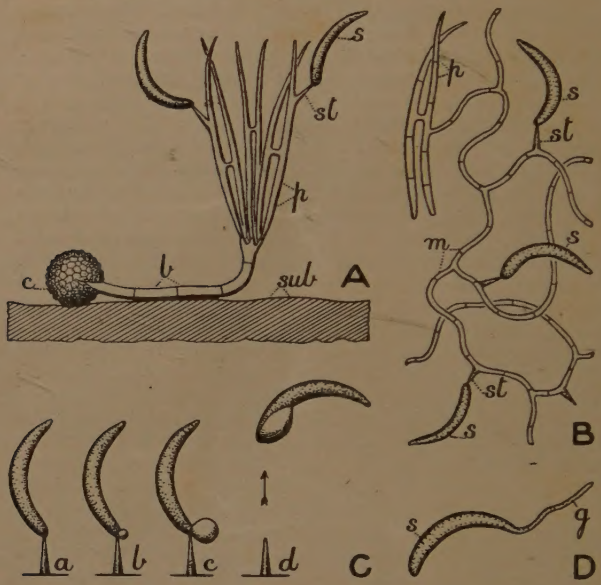


FIG. 1.—Diagrams illustrating spore-production in *Tilletia Tritici*. A: a chlamydospore *c*, with its basidium *b* crowned by six primary conidia *p*, which have conjugated in pairs; two of the pairs have produced short sterigmata *st* terminated by sickle-shaped secondary conidia *s*; *sub*, a damp substratum. B: a pair of conjugated primary conidia *p* on malt-agar; their germ-tube has developed into a branched mycelium *m*, which at intervals sends up into the air short sterigmata *st*, which bear sickle-shaped secondary conidia *s*. C: stages in the discharge of a single secondary conidium; *a*, a full-grown spore on its sterigma; *b*, the same with a drop of fluid just appearing at the spore-hilum; *c*, the same, about 20 seconds later; *d*, the same, one second after *c*, the spore and the drop have been violently shot from the sterigma. D: a discharged secondary conidium *s*, which has fallen on malt-agar and has germinated; *g*, its germ-tube. Magnification: A and B, 300; C and D, 400.

sterigma to the end of which it is attached in an asymmetrical manner. As in the basidiospores of mushrooms and rust fungi, the axis of the basal part of the conidium is inclined to the axis of the sterigma at an angle of about 45°. Hitherto, no one seems to have suspected that the sickle-shaped secondary conidia are the basidiospores of the stinking smut fungus.

One of us (A. H. R. B.) was struck by the great

resemblance of the sickle-shaped secondary conidia and their sterigmata to the basidiospores and sterigmata of the Hymenomycetes (mushrooms and toadstools),[4] Uredineæ (rust fungi),[5] and the three species of the recently described basidiomycetous yeast-genus Sporobolomyces ;[6] and it was therefore thought probable that these smut conidia, like the basidiospores just mentioned, are shot away from their sterigmata with the accompaniment of drop-excretion at the spore-hilum. The other author (T. C. V.) of the present communication undertook to test this supposition, with the result that, after overcoming the technical difficulties associated with (1) the germination of the chlamydospores in sterile 4 per cent. malt-agar and with (2) the development of the secondary conidia under conditions in which they could be watched for hours under the microscope, he made preparations which have enabled both of us to witness the violent discharge of the secondary conidia, with the accompaniment of drop-excretion. Since 1883, the year in which Brefeld began to publish his classical researches on the smut fungi, many mycologists, phytopathologists, and general botanists have studied these organisms ; but, hitherto, the fact that a smut fungus should display violence in the discharge of its spores has not been observed and does not even seem to have been suspected by any one.

A sickle-shaped spore, under laboratory conditions, takes about an hour and fifteen minutes to develop from a tiny rudiment to full size and to mature. At the end of this time, if all goes well, a tiny drop of fluid begins to appear on the spore-hilum at the base of the spore just above the sterigma (Fig. 1, C). The drop grows visibly, protruding laterally, and, in about 20 seconds, attains a diameter equal to about one and one-half times the thickness of the spore. Then, suddenly, the spore and the drop are shot away from the sterigma to a distance of about one-half a millimetre. The sterigma left behind is apparently unchanged. Under suitable conditions, a thin spore-deposit of discharged spores gradually accumulates. On 4 per cent. malt-agar a spore begins to germinate within an hour of discharge from its sterigma (Fig. 1, D).

Sometimes abnormalities in spore-discharge may be observed. (1) A spore may grow to full size in the usual time, but there may be no drop-excretion at the hilum and no discharge. One spore remained seated on its sterigma unaltered for three days. (2) A spore

[4, 5] Cf. A. H. R. Buller, "Researches on Fungi," London, vol. 3, 1924, Fig. 204, p. 505.

[6] A. J. Kluyver und C. B. van Niel, "Über Spiegelbilder erzeugende Hefenarten und die neue Hefengattung Sporobolomyces," *Centralb. f. Bakteriologie*, Abt. 2, Bd. 63, 1924–1925, pp. 1-20, Taf. I and II.

may grow to full size and then, within an hour, may germinate at its apex. Such a spore never excretes a drop of fluid and is never violently discharged. (3) A spore may grow to full size and then, within an hour, a drop may be excreted at the spore-hilum; but the drop and spore are not discharged. Instead, the drop may run up the spore and then grow to an abnormally large size about the middle of the spore. The drop may dry up slowly and then the spore, while still seated on its sterigma, may germinate by sending out a germ-tube at its apex. The failure of the drop to be excreted and excessive drop-excretion, accompanied by failure of the spore to be discharged, as just described, have been observed by one of us as not infrequent abnormalities of the spore-discharge mechanism both in the Hymenomycetes and the Uredineæ.[7] Also in *Puccinia graminis* the same author has observed that a basidiospore, which has failed to be shot away, often germinates *in situ* at its apex.[8] Thus the abnormalities connected with the phenomenon of spore-discharge in the secondary conidia of our stinking smut fungus find their exact parallels in the abnormalities one may witness in a mushroom or in the fungus which causes the black stem rust disease of wheat.

Our discovery that the sickle-shaped spores or secondary conidia of Brefeld and others are violently shot away from their sterigmata, with accompanying drop-excretion at the spore-hilum, is of considerable theoretical importance; for it not only confirms with new and weighty evidence the correctness of the view generally held by botanists that the Tilletiaceæ belong to the great group of the Basidiomycetes, but it also allows us to draw the following new conclusions: (1) the so-called secondary conidia of *Tilletia Tritici* and of other species of Tilletia are in reality the true basidiospores; and (2) the sporidia or primary conidia of Brefeld and others are morphologically equivalent to sterigmata. Finally, it seems to us probable that further research will show that our new conception of the basidium in the genus Tilletia may be successfully applied to the basidia of the other genera of the Tilletiaceæ.

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[7] A. H. R. Buller, "Researches on Fungi," vol. 2, 1922, pp. 18, 308-310; vol. 3, 1924, pp. 506-509.
[8] *Ibid.* vol. 3, Fig. 203, p. 503.

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